Successful Use of a Physiologically Acceptable Artificial Skin in the Treatment of Extensive Burn Injury

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A bilayer artificial skin composed of a temporary Silastic epidermis and a porous collagen-chondroitin 6-sulfate fibrillar dermis, which is not removed, has been used to physiologically close up to 60% of the body surface following prompt excision of burn wounds in ten patients whose total burn size covered 50–95% body surface area (BSA). Following grafting, the dermal portion is populated with fibroblasts and vessels from the wound bed. The anatomic structure of the artificial dermis resembles normal dermis and serves as a template for the synthesis of new connective tissue and the formation of a "neodermis," while it is slowly biodegraded. This artificial skin has physiologically closed excised burn wounds for periods of time up to 46 days before the Silastic epidermis was removed. At the time of election when donor sites are ready for reharvesting, the Silastic epidermis is removed from the vascularized artificial dermis and replaced with 0.004 autoepidermal graft in sheet or meshed form. Clinical and histologic experience in a relatively short follow-up period (2–16 months) indicates that "neodermis" retains some of the anatomic characteristics and behavior of normal dermis, thus promising improvement in the functional and cosmetic results, as well as providing physiologic function as a skin substitute. The artificial skin is easily sterilized and stored at room temperature, capable of large scale production, and immediately available for grafting, indicating its potential for easy and relatively economic use in the burn patient.

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THE TREATMENT OF EXTENSIVELY burned patients is a difficult clinical problem not only because of the extent of the physiologic abnormalities caused by the burn itself, but also because of the small area of normal skin available to provide replacement of the large area of skin destruction, which must take place if the patient is to survive the injury. There have been substantial improvements in the physiologic management of burn shock, infection, and metabolism which have considerably improved acute burn management. However, physiologic replacement of destroyed skin has not kept pace with the improvement in systemic management so that the burn illness is greatly complicated by the persistence of a large, open wound. If this wound is not promptly closed, malnutrition and bacterial invasion set the stage for extensive complications and a high mortality rate. To reduce the extent and duration of open wound, measures such as the use of allograft in moderate and large injuries,[1] and temporary transplantation and immunosuppression for massive injuries,[3] as well as technical innovations, such as autograft meshing,[10] are used and have improved prognosis following an extensive burn. Unfortunately, the full benefits of prompt excision of necrotic burned tissue and immediate wound closure (which would, at least on a theoretical basis, greatly simplify the therapeutic problems following burn injury) have not been realized because there is no immediately available and physiologically acceptable replacement for extensive areas of destroyed skin.

The need for a satisfactory, permanent physiologic replacement of skin has long been recognized, and extensive work has been performed in the past attempting to develop synthetic skin. Work in the past, for a large part, has concentrated, without striking success, on organic polymers with an emphasis on providing a membrane which was biologically inert, controlled fluid loss and bacterial entry, and which adhered to the wound.

In the authors' attempt to provide an acceptable artificial skin, the concept of nonreactivity in tissue was abandoned. An attempt was made to design a material with biochemical, mechanical and physicochemical properties intended not only to optimize physical and chemical properties, such as surface energy, modulus of elasticity, energy of fracture, and

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TABLE 1. *Properties Included in Design Criteria for Bilayer Artificial Skin*

Physical Properties	Biologic Properties
Moisture flux and fluid loss	Controlled rate of biodegradation
Bending rigidity	Non-toxic metabolites
Tear strength	Low or absent antigenicity
Modulus of elasticity	Absent inflammatory or foreign body reaction
Surface energy	Facilitate invasion of normal fibroblasts and capillaries
Peel strength	Synthesis of neodermal tissue
Epidermal portion impermeable to bacteria	Prevent infection
Control of pore structure in dermal portion	Modulate contracture
Handling and suturing characteristics	Modulate scar formation

moisture permeability, but in addition to provide a skin substitute that actively interfaces with the open wound surface in a nonantigenic, noninflammatory fashion, inducing the migration of normal fibroblasts and vessels into the material. The artificial material would act as a template for the synthesis of a new dermal matrix while controlling the rate of implant biodegradation in order to maintain the physical and biologic properties of skin necessary for physiologic wound closure. In short, the goal in this work was to provide a nonantigenic membrane closely resembling dermis in its anatomic structure and chemical composition, which would act as a biodegradable scaffolding inducing the synthesis of a "neodermis." The authors' hypothesis stated that the anatomic construction and chemical composition of the grafted artificial dermis would act as a model for the synthesis of a dermis-like structure whose physical properties would resemble dermis more than they resembled scar. The following describes the construction and clinical testing of this artificial skin in patients extensively burned.

Materials and Methods

Artificial Skin

The artificial skin described is a bilayer membrane made of distinct epidermal and dermal portions developed through extensive physicochemical and animal experiments over the past ten years.[13] The function of each portion physiologically resembles its counterpart in normal skin. The material is designed to contain physicochemical properties providing optimal "wetting" and "draping" properties, leading to elimination of dead space, surface adherence, control of bacterial invasion and fluid loss, while inducing cellular and vascular invasion, which would synthesize a dermal matrix while biodegrading the artificial implant. The criteria for artificial skin design are outlined in Table 1. The variables controlled to provide the required physical and biochemical properties of the artificial skin are listed in Table 2. The following describes the manufacture of the dermal and epidermal portions of the artificial skin.

TABLE 2. *Variables Controlled to Provide Required Physical and Biochemical Properties of Artificial Skin*

Content of chondroitin 6- sulfate (GAG)
Pore structure
Crosslink density
Retention of "native" collagen triple helix structure
Resistance to collagenase degradation

TABLE 3. *Flow Diagram for Manufacture of Dermal Portion of Artificial Skin*

Collagen dispersion + Chondroitin 6- sulfate solution $\xrightarrow[\text{pH } 3.2]{\text{Coprecipitation}}$

Casting of fibrillar precipitate in pans $\longrightarrow$ Freeze drying $\longrightarrow$

High temp vacuum dehydration crosslinking (105°C.) $\longrightarrow$ Glutaraldehyde crosslinking

Elution of aldehyde $\longrightarrow$ Freeze drying

Dermal portion. The raw material used to manufacture the dermal portion is a preparation of bovine hide collagen (donated by the Eastern Regional Research Center, U.S. Department of Agriculture, Philadelphia, PA) and chondroitin 6-sulfate obtained from shark cartilage (sodium salt, Type C, Sigma Chemical, St. Louis, MO), as generally outlined in the diagram in Table 3. Physiochemical, biochemical, and mechanical properties[14] are controlled by the content of chondroitin 6-sulfate, methods of cross linking and cross link density and mean pore size.[16] The dermal portion of the artificial skin was sterilized in manufacture utilizing the two bactericidal steps of heating to 105 C[9] followed in a subsequent step by immersion in glutaraldehyde solution 0.05 wt. %,[12] which produced a bacteria-free membrane, as documented by extensive bacteriologic survey.

Epidermal portion. The epidermal portion of the artificial skin consists of a homogeneous layer of medical grade Silastic (Dow Corning) approximately 1/10 mm thick. This material controls water flux from the dermis to approximately equal than of normal skin. Liquid Silastic is applied in bacteria-free fashion to the sterile artificial dermis, making a firm bond to the artificial dermis as it cures. This provides an intact,

sterile, bilayer artificial skin consisting of an epidermal and dermal portion.

The sterile artificial skin is stored in sealed polyethylene bags either as a freeze-dried, bilayer membrane, or in 70% isopropyl alcohol. For ease of handling and use in these initial clinical trials, the isopropyl alcohol procedure has been used for packaging and storage before clinical application. In either case, the shelf life of the artificial skin is an extensive period of time at room temperature conditions on open shelf storage.

Clinical Evaluation

Ten patients, 3–60 years old, whose third degree burns extended from 50 to 90% of their body surface area (BSA), and who had an extensive portion of total body surface closed with artificial skin, following informed consent, are reported. Patients with less than 15% BSA closed with artificial skin are excluded.

All patients were treated with prompt excision and immediate closure of their burn wounds. Complete excision of all full thickness and deep dermal injury was carried out in stages and completed by day 10 following admission to our burn service. Each excisional procedure was limited to 15–20% BSA. In the initial excisional procedures, the wound was closed with meshed autograft harvested at the same operative procedure. Following the use of all available autograft, excised wounds were closed with artificial skin immediately following excision. The distribution of the body surface burned and the areas of artificial skin application are recorded for each patient in Figure 1.

Method of Artificial Skin Grafting

The techniques used to graft artificial skin are essentially the same as those used for autografting an excised burn wound.[2] Following excision of burn eschar (Fig. 2a) which, in the patients reported, was carried out both to the level of the fascia and sequentially into the deep dermis or subcutaneous tissue, meticulous hemostasis was obtained and artificial skin in sheets measuring 4 × 6 inches was tailored to fit the existing open wound and sutured in place, using interrupted sutures of 5-0 chromic catgut (Fig. 2b). Great care was taken in constructing the suture lines between unburned skin and artificial skin to achieve primary closure. Wide sheets of the artificial material could only be used over large, relatively flat areas such as the chest, abdomen, back, or upper thighs. Narrower strips were used on the forearms and legs, and over joints in order to avoid wrinkling. Care was taken to prevent wrinkling by suturing the artificial skin under slight tension. If, however, wrinkles developed in the material in the postoperative period, the ridge of the wrinkle was debrided in the patient's room in order to drain the collection of serous material which inevitably collected underneath. The adherence of the artificial dermis to the underlying wound was comparable to that of autograft, and firm fixation of artificial dermis to wound bed was achieved within minutes of application. The wounds closed with artificial skin were treated in the same fashion as allograft or autograft areas, using open technique on the anterior chest and abdomen, and quilting technique on the back, neck, and extremities. Vascularization of the artificial material was evident by blanching on pressure in three to five days.

The artificial dermis was never removed, serving as template for ingrowth of host cells and vessels synthesizing a neodermis as the original artificial dermal scaffolding was slowly biodegraded and removed. The Silastic epidermis, on the other hand, was a temporary measure, being replaced by autoepidermis at a time of clinical convenience. When autograft donor sites were suitable for reharvesting, the patient was returned to the operating room and donor sites reharvested with the dermatome set at 0.004 inches, in order to obtain a very thin graft containing primarily epidermis with a scant amount of dermis. The Silastic epidermis was then easily peeled from the artificial dermis (Fig. 2C) leaving a partly replaced vascularized "neodermis" with a regular granular surface whose color was slightly yellow to red, depending on the length of time between initial grafting and removal of this Silastic epidermis. A small amount of capillary bleeding was easily controlled using saline packs. The "neodermal" wound was then closed with the thin epidermal autograft. Both sheet and three-to-one meshed grafts were used (Fig. 2d) The epidermal grafts were sewn into place and dressed in a conventional manner.

Each area of artificial skin graft was carefully followed with clinical evaluation and a biopsy specimen was obtained at the time of Silastic epidermal removal and epithelial grafting.

Histology and Immunofluorescence

Biopsy specimens of artificial skin were fixed in 10% formaldehyde, processed in the Autotechnicon, embedded in paraffin, sectioned to 6 μm and mounted on glass slides. They were stained with hematoxylin and eosin, Masson trichrome, or the indirect immunofluorescence technique described below.

Preparation of Human Type IV Collagen

Human Type IV (basement membrane) collagen was prepared from cadaver kidneys by the heat gel fractionation method, described by Trelstad.[11]

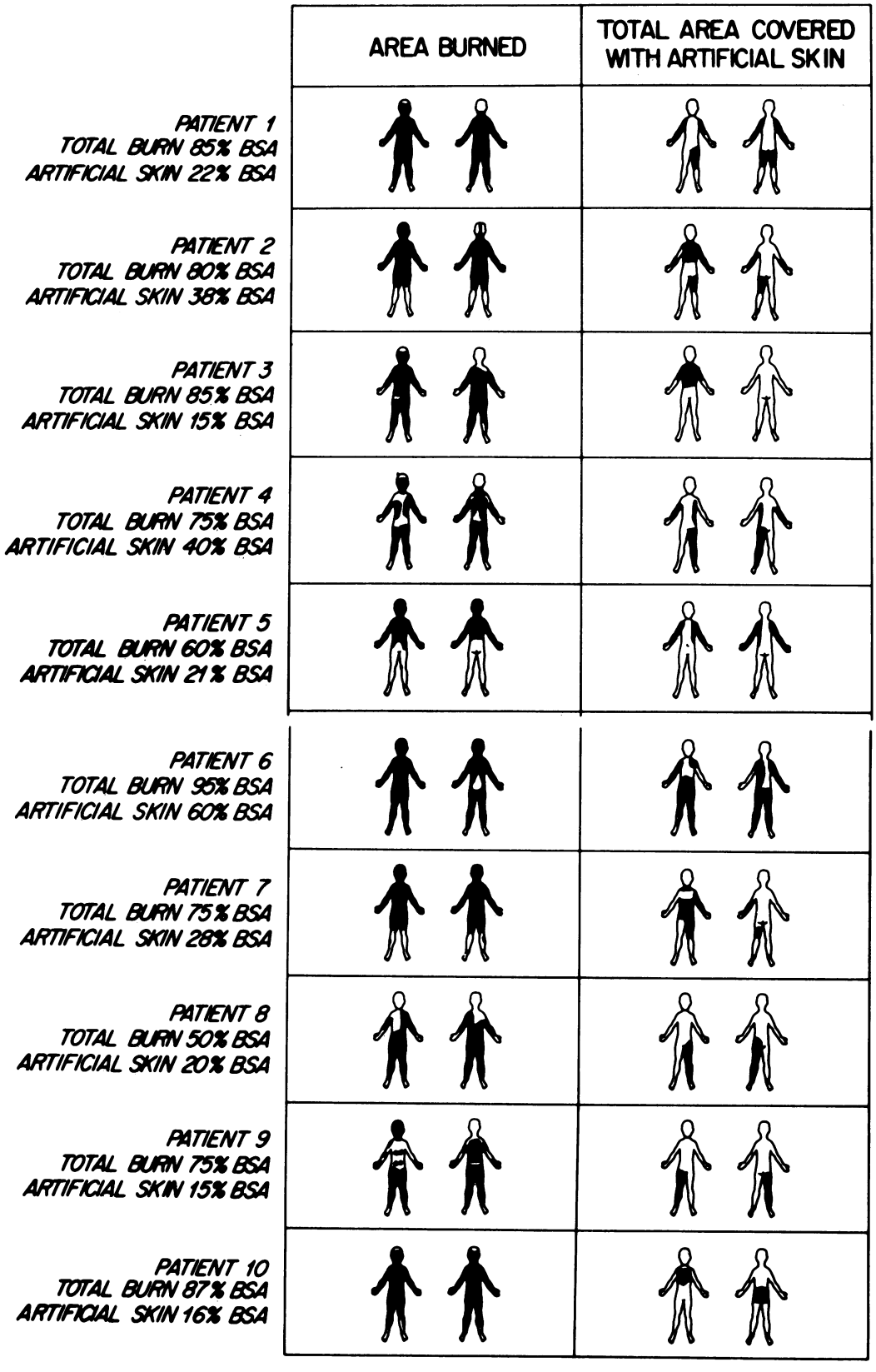

FIG. 1. Characteristics of patients and areas of treatment with artificial skin.

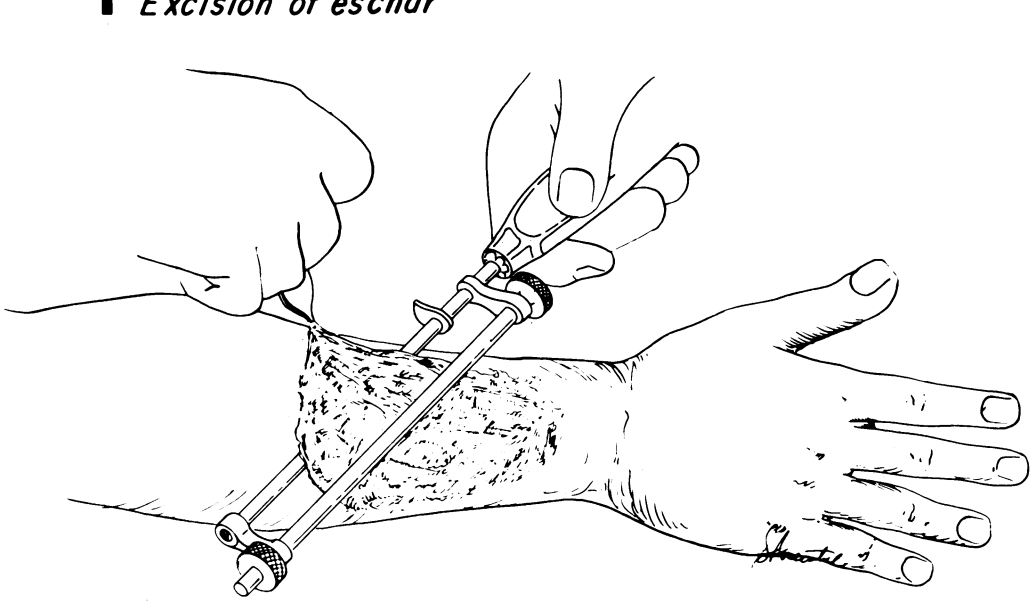

FIG. 2a. Excision of all necrotic tissue using a guided knife, followed by careful hemostasis.

Antiserum

Rabbits were immunized subcutaneously with an emulsion of 1–2 mg Type IV human collagen and complete Freund's adjuvant (Difeo). Booster emulsions were prepared with incomplete Freund's adjuvant, and administered subcutaneously two weeks later, and at eight week intervals thereafter. Blood which was collected ten days following booster injections via arterial–ear puncture was clotted and the serum stored at 20 C until used. Specificity of this antisera was ascertained by indirect immunofluorescence of frozen section and formalin-fixed paraffin-embedded tissue sections and by absorption with Types I, II, III, and IV collagens. These antisera were found to be monospecific for the basal lamina of skin, muscle, epithelial derived parenchyma, blood vessels and capillaries of a variety of organs and tissues. There was no cross

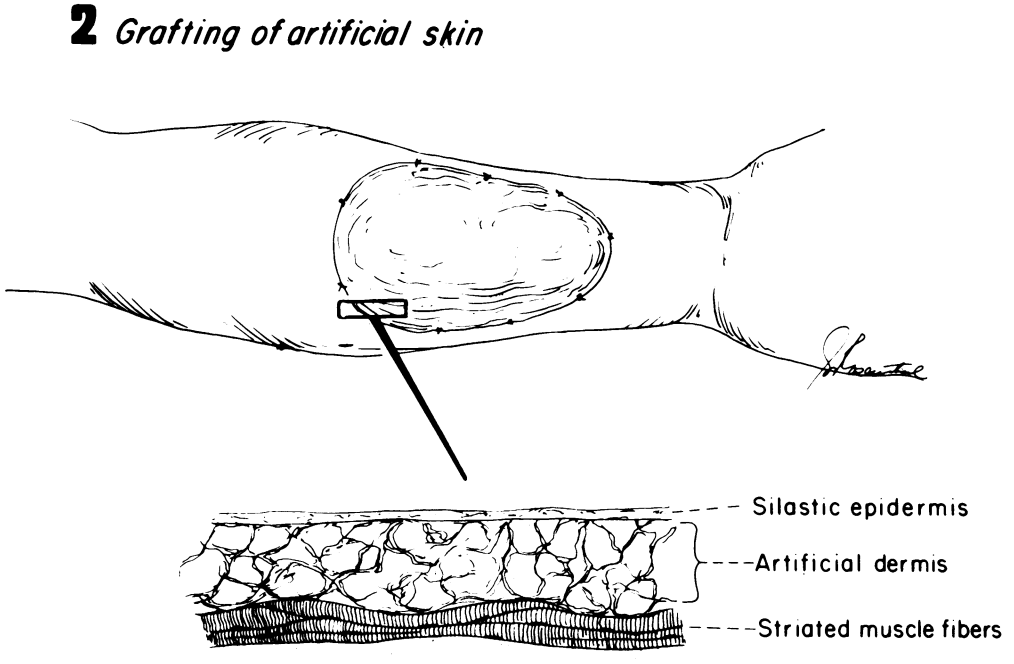

FIG. 2b. The artificial skin is grafted using conventional techniques and carefully sutured to achieve primary closure. The insert shows that the artificial dermis adheres to the excised bed, whether dermal remnants, subcutaneous tissue, facia or muscle, without dead space or fluid collection.

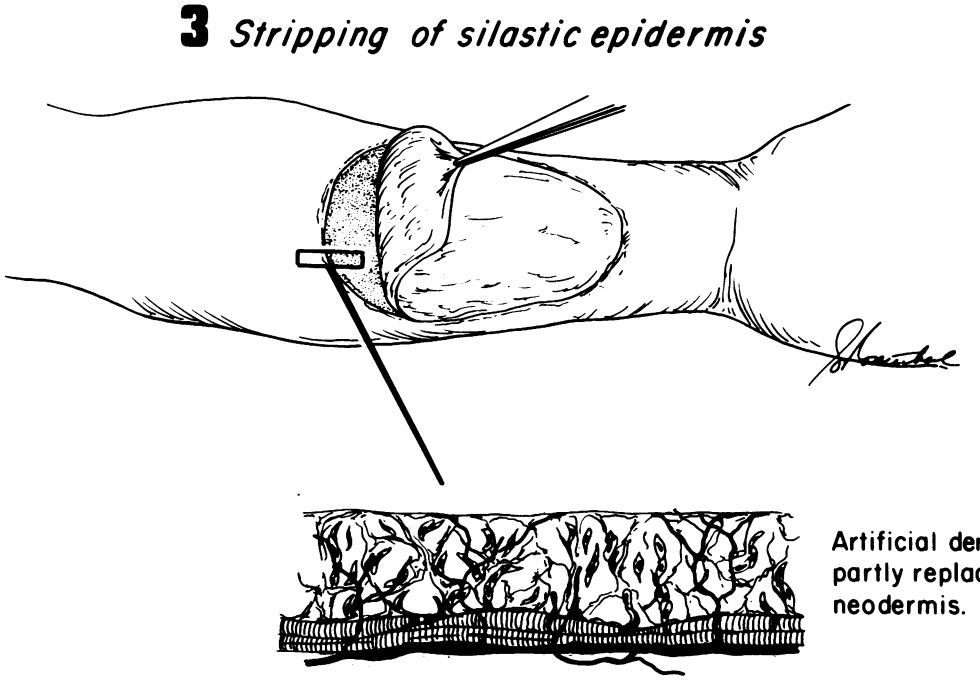

FIG. 2c. At the time of election, when the donor sites are ready for re-harvesting, the Silastic epidermis is easily stripped from the artificial dermis using forceps. Insert shows that the artificial dermis is invaded by host cells and vessels and that it is partially replaced by a newly synthesized "neodermis."

reactivity to intracellular organelles or other extracellular substances, collagens or elastin.

Immunofluorescent Stain

Staining techniques for immunofluorescence were used as described by Huang[7].

Results

Ten severely burned patients treated with staged, prompt eschar excision and immediate wound closure performed shortly after admission to the Massachusets General Hospital or the Shriners Burns Institute in Boston, and who had 15% of more of their excised

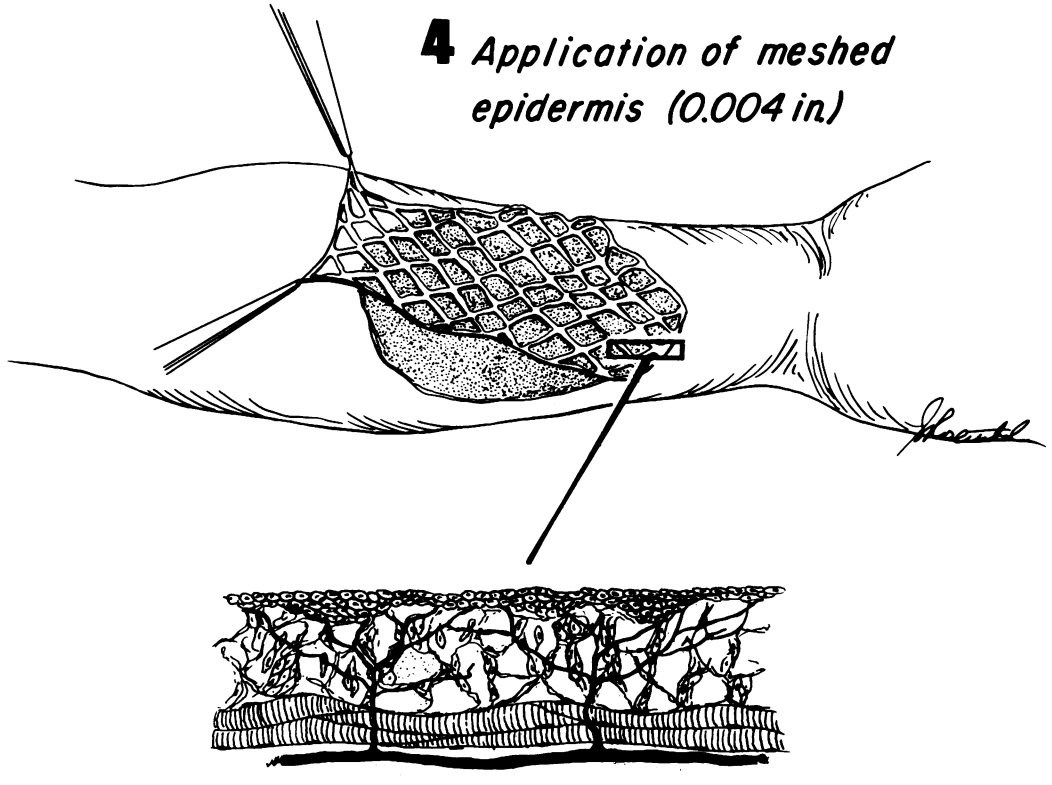

FIG. 2d. Photomicrograph demonstrates grafting of the "neodermis" with a thin epidermal graft directly onto the dermal bed provided by the artificial dermal template.

wounds closed with artificial skin, are included in this report. Table 4 gives the general characteristics of these patients. Their total burn size ranged from 50 to 95% BSA (average: 77% BSA), and third degree burn size ranged from 50 to 90% BSA (average: 64% BSA). The patients ranged in age from three to 60 years (average: 36 years). There were seven males and three females included in the study. All the patients had received flame burns.

The artificial skin was grafted into the excised burn wound, as previously described, in a careful attempt to achieve primary closure. Table 5 outlines the general characteristics of these artificial skin grafts as applied to extensive burn wounds. In the ten patients treated, the total area closed with artificial skin ranged from 15 to 60% BSA (average: 27.5% BSA), and the time the wound was closed with the intact artificial material (*i.e.*, before removal of Silastic epidermis for the replacement with autoepidermis) was 14–46 days (average: 26 days). In these patients, no artificial skin was lost secondary to infection, nor did any infection develop in or adjacent to the artificial skin grafts.

There were three untoward complications associated with the artificial skin grafting. First, a loss of artificial skin occurred over approximately 3% of the body surface area secondary to hematoma developing under the graft at the time of graft placement in one patient. Second, several patients developed shallow wrinkling of their artificial skin graft within a day or two of grafting, particularly over the arm or lower leg, usually associated with application of large areas of artificial skin. These wrinkles developed a sterile, serous exudate. The wrinkle crest was easily debrided with scissors on the ward to avoid widening of the area of nonadherence. This left a strip of open wound 1–2 mm in diameter. None of these debrided wrinkles became infected, nor did they cause any difficulty in later epidermal grafting. A third problem infrequently occurred, usually two to three weeks following grafting. At this time, particularly in areas of wear or motion, the edge of the Silastic epidermis became loosened from the dermal bed. In no case did the artificial dermis separate from the wound bed. These areas of loosened Silastic epidermis were promptly debrided back to a point of firm adherence between artificial epidermis and dermis to prevent further separation.

TABLE 4. *Characteristics of Patients Receiving Artificial Skin*

Number of patients	10
Total burn size	50 to 95% BSA (average 77% BSA)
3° burn size	50 to 90% BSA (average 64% BSA)
Age	3 to 60 years (average 36 years)
Sex	7 males and 3 females
Type of burn	Flame—all patients

TABLE 5. *Characteristics of Artificial Skin Grafts*

Total area closed with artificial skin	15 to 60% BSA (average 27% BSA)
Time of wound closure using silastic epidermis	14 to 46 days (average 26 days)
"Take" of artificial skin on excised wound bed	95 to 100%
"Take" of autoepidermis on "artificial dermis"	85 to 95%
Artificial skin loss secondary to sepsis	None
Complications	Artificial skin loss (3% BSA) secondary to hematoma under graft (one patient)
Follow-up	2 to 16 months
Long-term clinical, cosmetic and functional results	Good to excellent

This complication left narrow, irregular edge areas of artificial dermis exposed to dessication. In these narrow areas, never amounting to more than 5–10% of the grafted area, epidermal graft take was less efficient than over areas continually protected by Silastic epidermis.

Overall, the take of artificial skin on the excised wound bed approached 100%, and the take of autoepidermis on artificial dermis immediately following removal of the Silastic epidermis ranged from 85 to 95%. Healing of the epidermal grafts onto the vascularized artificial dermis was rapid, and the thin epidermis rapidly thickened to what appeared to be normal epidermal thickness, in ten days to two weeks. In the patients treated with meshing of the thin autoepidermal sheets, filling in of the interstices proceeded at the rate expected from conventionally harvested and meshed autografts which contain not only epidermis but substantial thickness of dermis as well. A striking observation concerning the healing characteristics of epidermis grafted on vascularized artificial dermis is the character of the healing pattern itself. Even early after complete epidermal closure, very little evidence of a meshing pattern was detectable, and after several months, the pattern of mesh could only be detected on very careful observation. In addition, the donor sites following harvesting, with the Paget dermatome set at 0.004 inches, were re-epithelialized within a week after harvesting, even in areas which had been harvested for the second or third time.

The general clinical observations concerning healing with lack of inflammation contracture or rejection with maintenance of elasticity and suppleness in the healed wound are confirmed by histologic evaluation of biopsy specimens obtained from the grafted artificial skin. Biopsy specimens were obtained at various stages of

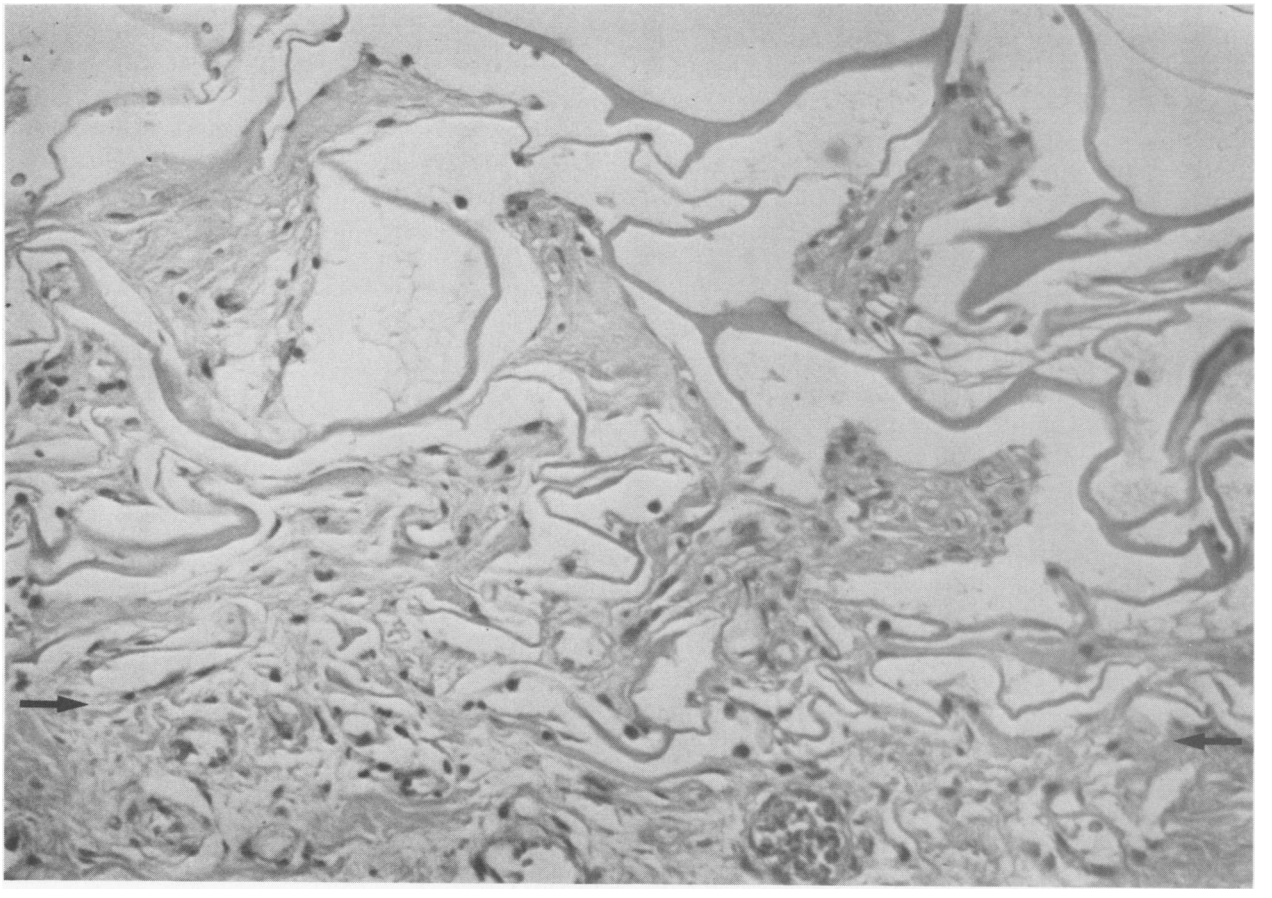

FIG. 3. Photomicrograph of artificial dermis with tufts of newly synthesized connective tissue matrix migrating into the artificial collagen lattice. The junction between artificial dermis and host bed is marked by an arrow. Biopsy taken one week following artificial skin grafting.

healing from one week to six weeks following excision and artificial skin grafting and an additional three weeks following removal of Silastic epidermis and epidermal grafting.

Biopsy specimens of grafted artificial skin were examined with a light microscope with H & E staining and showed a full range of host growth patterns. This progression was arbitrarily divided into three phases—early, intermediate and late—which correspond to growth of connective tissue occupying successive thirds of the implanted artificial dermis.

In the early phase (Fig. 3), small dome-shaped tufts of young connective tissue bud into lower lattice pores from the host–graft bed. The cellular tufts consist of large mononuclear cells, spindle cells, small open spaces, capillaries, and an eosinophilic fibrillar matrix. The mononuclear and spindle cells have no discernible cytoplasmic borders, and appear as naked nuclei embedded within the fibrillar matrix. These cells appear to represent primitive mesodermal cells and the more differentiated cells of the histocytic-fibroblastic series. Small open spaces and definite endothelial lined vessels permeate these tufts. Some capillaries contain red blood cells. The fibrillar matrix is not birefringent and does not stain positive for collagen. The underlying wound bed is remarkable in its absence of inflammatory or foreign body reaction.

In the intermediate (Fig. 4) and late phase (Fig. 5) these tufts are expanded and fill the lower lattice spaces. In these later phases this neoconnective tissue shows less cellularity, increased matrix deposition and increased numbers of well developed blood filled vascular channels. The matrix now shows birefringence and is Masson trichrome positive for collagen.

The tissue interface between the artificial dermis and the host bed was variable in thickness at five to six weeks after artificial skin grafting. Some foci showed moderately thick zones of vascular-fibrous connective tissue (Fig. 5), while in other foci the transition from host skeletal muscle or fat to lattice was abrupt, with little intervening fibrous stroma (Fig. 6).

The long-term status following removal of Silastic

FIG. 4. Photomicrograph of grafted artificial skin 14 days after grafting. There is extensive infiltration of cells, matrix and vessels into the artificial dermal lattice extending into the inner two-thirds of the artificial dermis.

epidermis is shown in (Fig. 7). The biopsy specimen was obtained from a patient whose artificial skin was grafted for four weeks before the Silicone membrane was removed and a 0.004 inches epidermal autograft applied. A biopsy specimen obtained three weeks later showed a well healed epidermal covered skin. No remnants of the bovine collagen lattice are detectable. There is complete remodeling and the dermis is morphologically indistinguishable from moderately fibrotic dermis.

Artificial skin implants in the intermediate or late phase showed numerous bright linear fluorescent staining of various size tubular structures throughout the budding and growing connective tissue cores (Fig. 8). Stain localization was correlated with basement membrane structures of capillaries, blood-filled vasculature, and other vascular channels.

Figure 5 is a photomicrograph of a biopsy specimen of artificial skin grafted five weeks before biopsy, at the time of Silastic epidermal removal. The patient had sustained a large, very deep burn, and the excision performed down onto the muscle of the upper arm. The histologic section shows that the grafted artificial dermal template has been converted into a tissue heavily populated with vessels, fibroblasts, and newly synthesized connective tissue matrix. We have called this "neodermis." At this stage, only small remnants of the artificial material remain. Immediately below the "neodermis" in focal areas is a layer of conventional scar secondary, we believe, to incomplete debridement of heat injured muscle. Other areas of the biopsy specimen show no scar between neodermis and muscle (Fig. 6). Below this scar is normal appearing striated muscle. Of special interest is the anatomic arrangement of the collagen bundles and vessels in the "neodermis" as contrasted to the layer of underlying scar. The newly synthesized collagen matrix has retained much of the open, loosely woven anatomic structure of dermis, differing from the usual heavy, solidly packed collagen bundles seen in conventional scar. This reproduction of anatomic structure resembling dermis has also retained some of the physiologic properties of dermis, as manifested clinically in elasticity, resilience, and absence of contracture in the time observed. Further, in all of the biopsy specimens obtained, no indication of even early de-

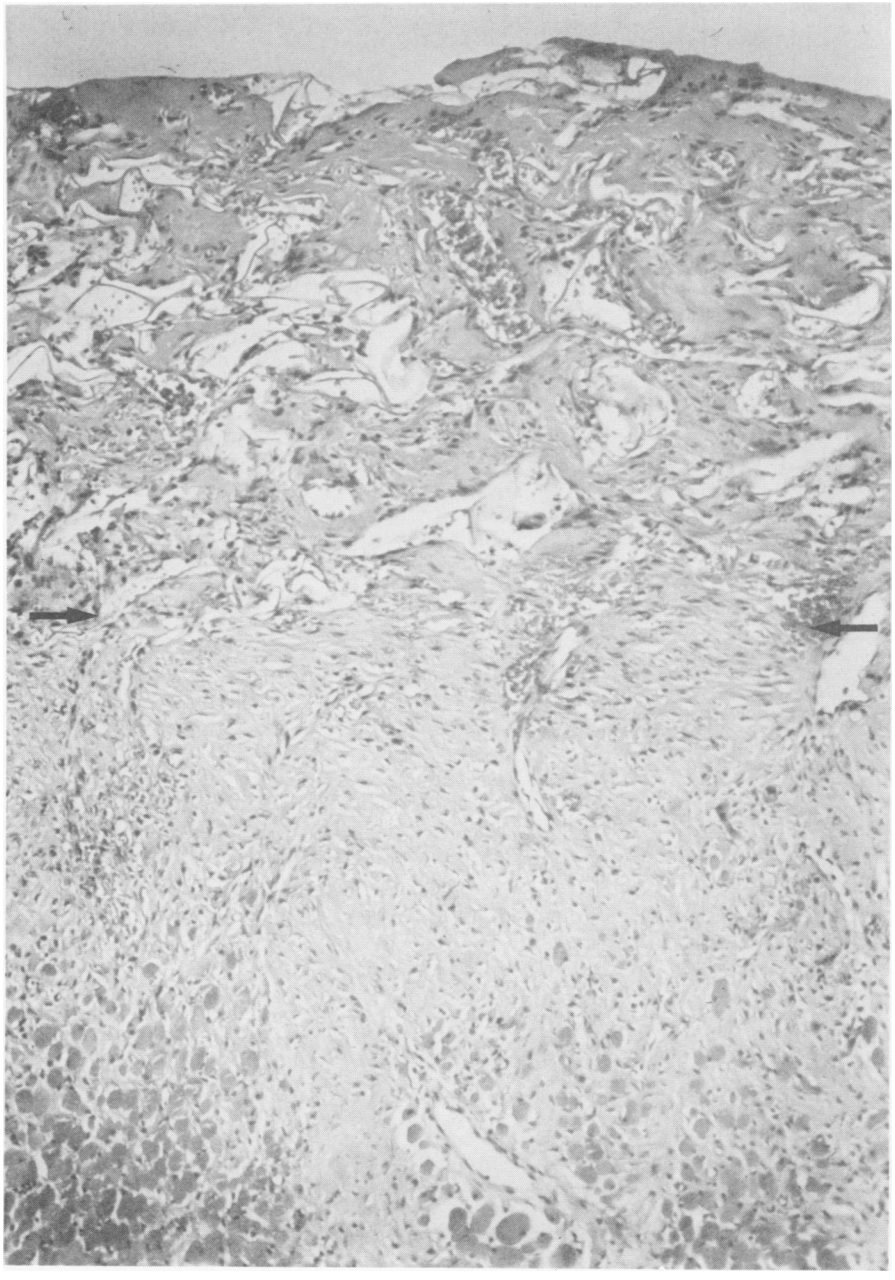

FIG. 5. Photomicrograph of artificial skin and host bed five weeks following implantation. The Silastic epidermis has been removed and the junction between artificial dermis and host bed is marked with arrows. There is extensive infiltration of connective tissue matrix and vessels throughout the entire width of the artificial dermis. There is a fibrous tissue scar between the artificial dermis in the underlying muscle. The artificial dermis has retained the loose characteristics of normal dermis, differing considerably from the scar in the muscle of the host bed.

velopment of hypertrophic scarring has been detected. All provide evidence indicating that the artificial dermis acts as a template for the reproduction of a "neodermis," which has important histologic and functional relation to normal dermis.

Long-term clinical evaluation of the results of early wound closure using artificial skin followed by epidermal grafting of the residual "neodermis" is limited by the small number of cases and the relatively short period of follow-up. In the series reported, the follow-up period ranges between two and 16 months. Clinical examination of the healed wound demonstrates that the grafted area quickly matures, losing its redness usually in weeks and presenting a smooth, homogeneous appearance that resembles normal skin. No evidence of hypertrophic scar formation or clinical contracture has been noticed in any of the patients treated, although there is an occasional area of reddened, thickened scar at the suture lines between sheets of artificial skin, especially over joints where the careful abutting of the two layers of skin has not been successful. Figure 9 compares the upper arms of a patient deeply burned whose excision extended to the deep fascia. The left upper arm and shoulder was covered with artificial skin followed by meshed epidermal graft 0.004 inches thickness, the right arm and

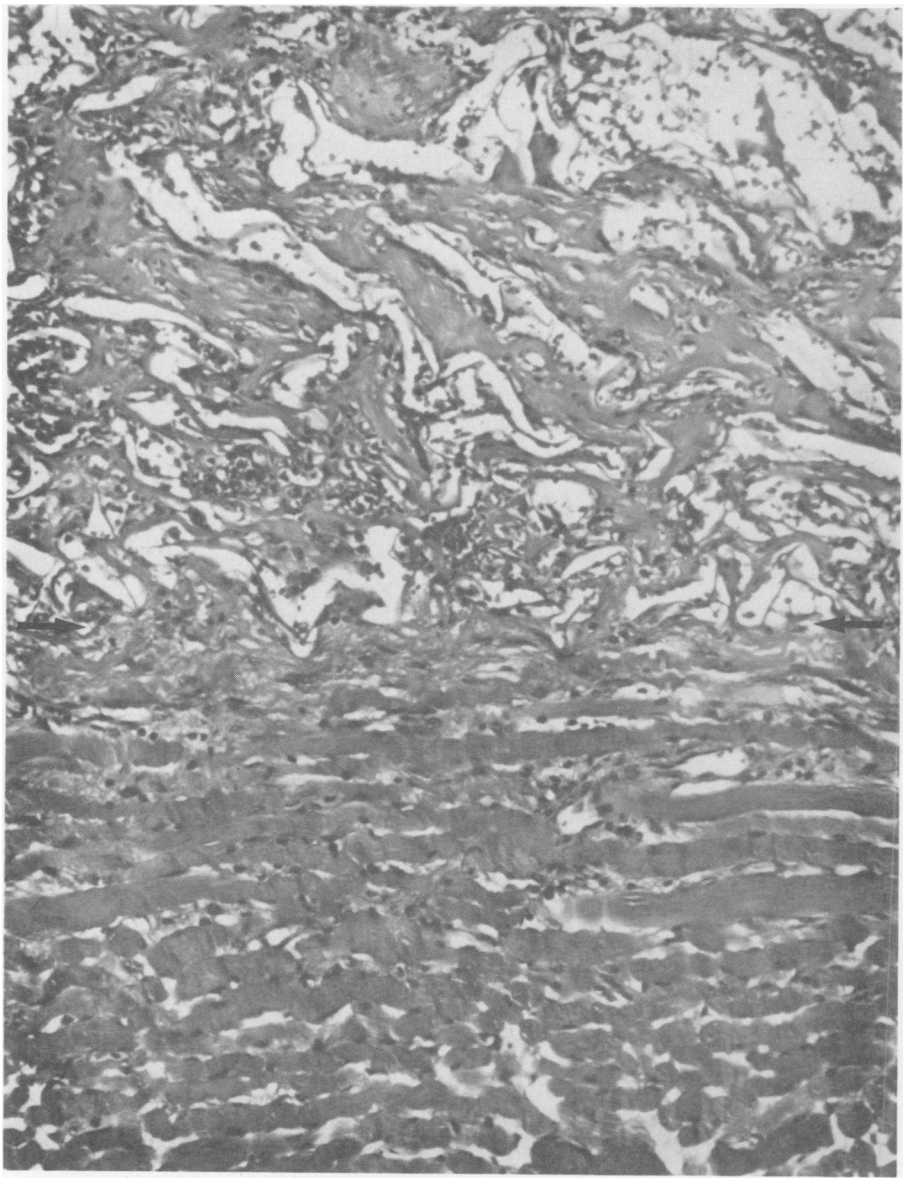

FIG. 6. Photomicrograph of the same artificial skin graft as shown in Figure 5, but from an adjacent area. The junction between artificial dermis and normal muscle is marked with arrows. There is no connective tissue scar present. The artificial dermis has healed directly without scarring to the underlying muscle.

shoulder was covered by conventional meshed autografting at 0.014 inches thickness. The appearance of the artificially grafted arm more closely resembles normal skin, and on palpation is found to be elastic, soft, and pliable. In contrast, the arm closed by conventional meshed autografting shows a prominent pattern of meshing with beginning hypertrophic scar in the areas of interstices. The skin feels stiff and thick to palpation without the normal skin resilience. The pictures represent artificial skin at two months and conventional meshed grafting at two and a half months following wound closure.

Discussion

The need for an artificial material capable of successfully substituting for skin has long been recognized. Although the need is most clearly seen in the treatment of extensive, full thickness burns, it is present in any disease or injury where large areas of the skin are destroyed. In order to meet this need, extensive work has been performed, concentrating on the use of the synthetic polymers[6] and collagen preparations.[5] For the most part, the search for synthetic polymers has concentrated on a nonreactive material which would adhere to open wound and possess the physical qualities capable of regulating fluid loss. All have been designed as a temporary wound closure and have not received wide acceptance.

This present work differs somewhat from previous concepts in that the authors' design attempts to achieve successful long-term use through an active interaction with the host tissue in the form of a non-

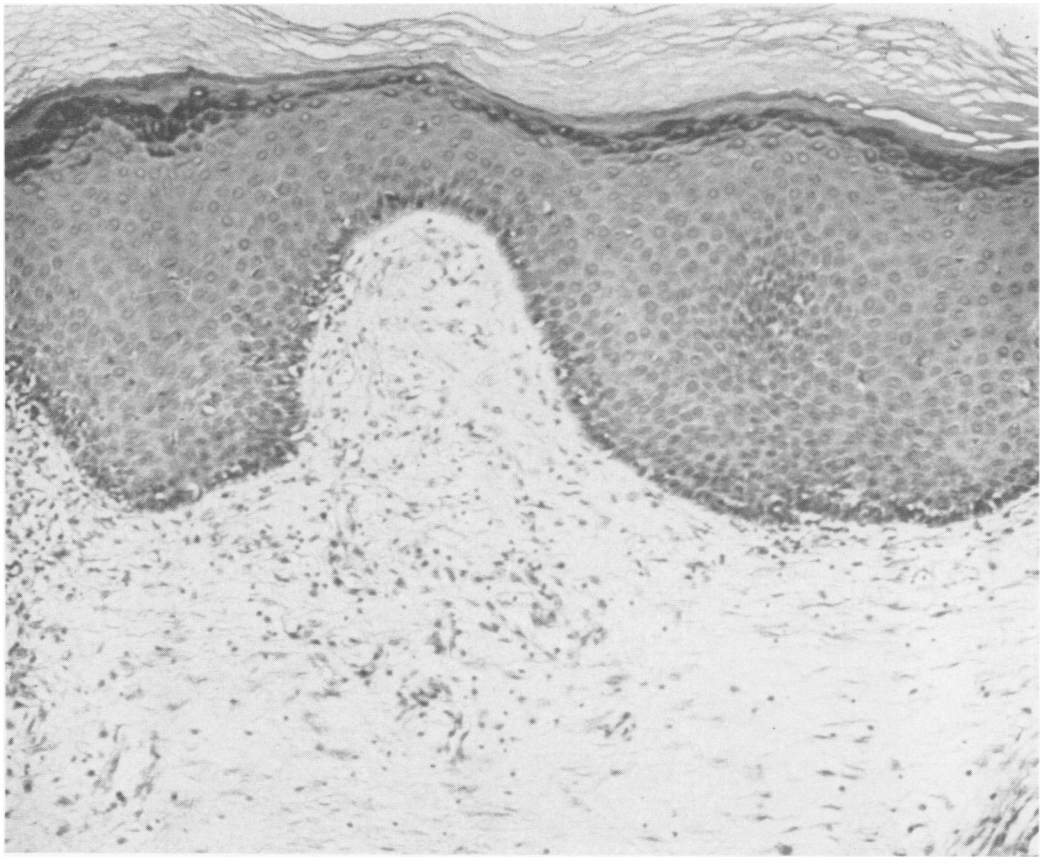

FIG. 7. Photomicrograph of well healed epidermis covering a "neodermis" provided by artificial dermal graft. The artificial dermis had been grafted four weeks before Silastic epidermal removal and the biopsy obtained three weeks following epidermal graft.

inflammatory membrane which acts as a biodegradable template for the synthesis of a permanent "neodermal" tissue. The biochemical, physicochemical and mechanical considerations required for a successful skin substitute seem best fulfilled by designing an artificial dermis capable of interfacing with the host tissue, and an epidermis capable of controlling water loss and bacterial invasion. The epidermal requirements were further divided into a temporary material accomplished by a thin layer of Silastic adequately controlling water loss and successfully providing protection from mechanical trauma and bacterial invasion. Long-term provision of epidermal function is carried out by autoepidermal cells, which are transplanted to the artificial dermis following vascularization. This can be accomplished by a number of maneuvers, ranging from growth of epidermal cells in tissue culture for later transplantation, which has been demonstrated in the authors' laboratory work as well as the work of others,[8] and the grafting of epidermal sheets as very thin split-thickness grafts harvested from the patient at the time of election. In this present clinical study, we have used the latter method.

Our most extensive development has been in the design of an artificial dermal template capable of adhering to an excised wound bed with an optimal modulus of elasticity, and strength, and with the ability to induce migration of normal fibroblasts and vessels which synthesize new connective tissue matrix while biodegrading the artificial material at a controlled rate.

An insoluble, native collagen preparation obtained from bovine hide, which had fully retained its triple helical structure, was used as a major component of the artificial dermis. Collagen was chosen because of its acceptable biologic characteristics, the production of nontoxic products on biodegradation, and because the extensive research on collagen has made it one of the best understood polymers, allowing us to use a complex but well-defined and reproducible material. In order to obtain optimal characteristics, the native collagen was precipitated in fibrils complexed to chondroitin 6-sulfate at pH 3.2. This complex is stabilized by what is believed to be covalent bonds by heating the freeze-dried material to 105 C under vacuum dehydration. Crosslinking of the collagen-chondroitin 6-sulfate coprecipitated material was central not only to immobilize the glycosaminoglycan content onto the collagen, thus preserving its resistance to biodegradation, but also to provide a

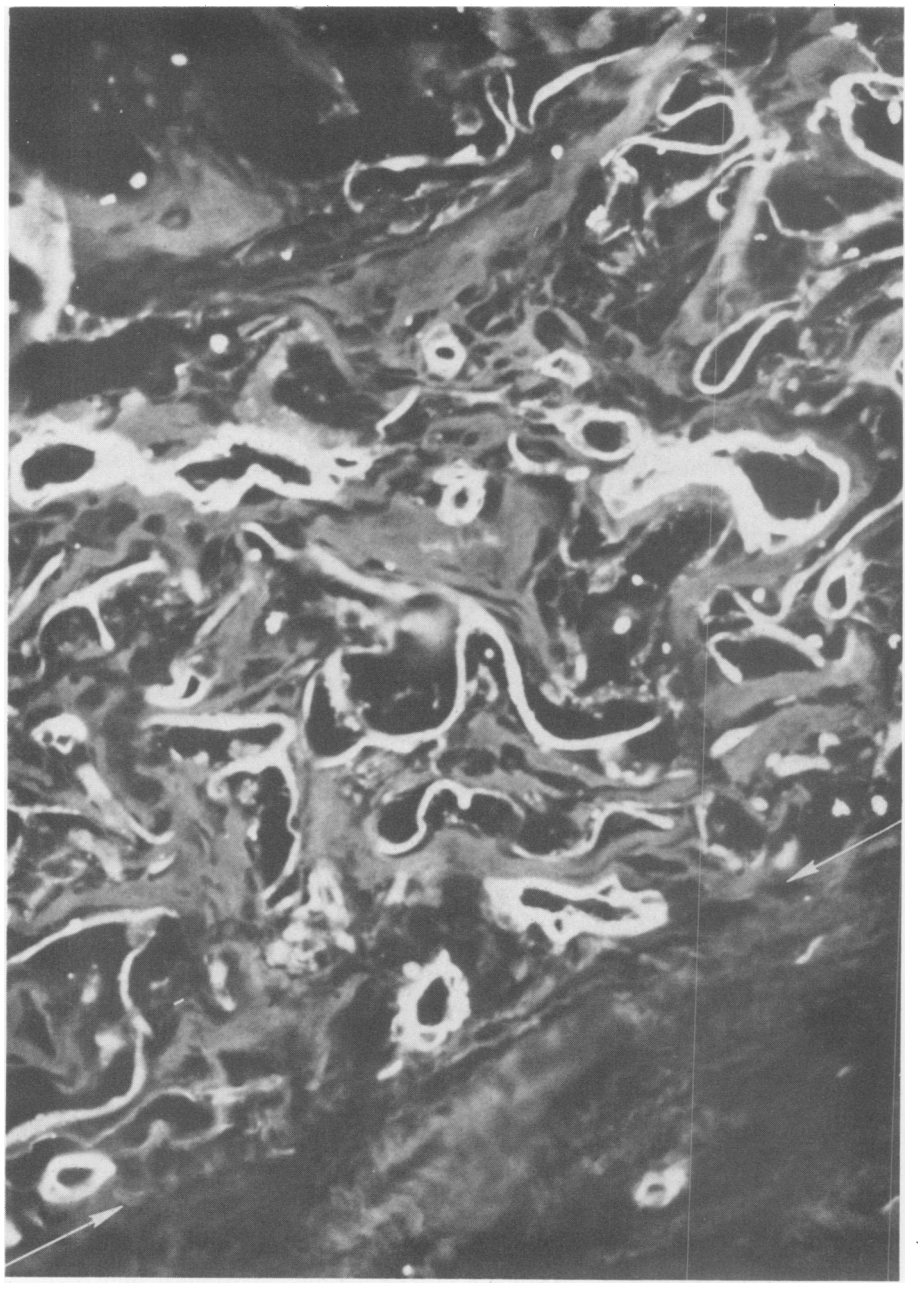

FIG. 8. Fluorescent micrograph of artificial dermis healed directly to underlying muscle. Junction between artificial dermis and muscle is marked with arrows. Heavy staining areas represent fluorescent marking localized on type four basement membrane collagen.

method of increasing tensile strength to a level where the membranes could be handled and sutured conveniently in a clinical context. Following a second crosslinking step with glutaraldehyde, the final crosslinked density of the material used clinically was on the order of 10,000 molecular weight between cross links. The collagen-chondroitin 6-sulfate fibrillar coprecipitate, therefore, provided important biologic properties in the way of controlling biodegradation, while at the same time maintaining a significantly higher modulus of elasticity and higher strength than collagen, which has been simply cross-linked. The addition of GAG plus crosslinking, therefore, not only controlled biodegradation but also gave useful mechanical behavior to the membrane.

In addition to the physicochemical properties of the material, it was quickly found that the morphologic characteristics of the fibrous, highly porous membrane produced by the distance between these fiber bundles, one from another, had a controlling effect on cellular and vascular populations of the artificial material. Pore sizes markedly smaller than the pore sizes found in normal dermis were found to retard cellular invasion and to promote a thick fibrous capsule sur-

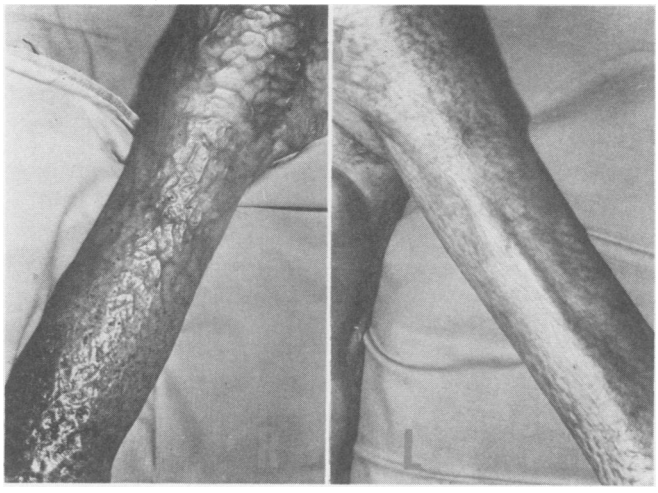

FIG. 9. Photographs of the left and right shoulder and upper arm of a patient receiving conventional 0.012 in. mesh grafting to the right arm and shoulder, seven weeks before photography, and artificial skin grafting to the left arm and shoulder seven weeks before photography followed by epidermal grafting three weeks before photography. There is a decrease in hypertrophic scar formation and of induration with less redness in the artificial skin grafted arm.

rounding the implanted artificial material. In addition, vascularization of the implant did not occur. We suggest that the pore structure of the collagen-GAG membrane must be very close in size to what cells and vessels encounter in normal dermis if optimal population of the grafted material is to take place. This has been achieved by a freeze-drying process which preserves the pore structure of the membrane almost intact.[6]

In extensive histologic study the light and fluorescent microscopic findings disclose that there appears to be a highly favorable host response to implantation and incorporation of this artificial skin. The host bed shows exuberant, very brisk growth of vascular connective tissue tufts into the spaces of the artificial dermal lattice. In a short period of time (one to two weeks) a highly vascular connective tissue matrix is produced and occupies the lattice spaces. At this point the implant appears to be a fertile tissue substrate for subsequent definitive epidermal grafting. This high vascularity was well demonstrated by the number of basement membranes containing vessels ascertained by immunofluorescent microscopic examination.

Light microscopic findings disclose no histologic evidence of host–graft rejection. An occasional lymphocyte or plasma cell can be found in the proliferating connective tissue cores or lattice spaces, but infiltration of lymphocytes or plasma cells, vascular changes, thrombosis, and tissue necrosis associated with graft rejection are not seen.

The collagen dermal lattice does not appear to have significant thrombogenic activity, as evidenced by absence of fibrin deposits in the lattice spaces or the proliferating connective tissue.

Microscopic examination of a clinically successful split thickness epidermal skin covering over an artifical implant disclosed a well-healed epidermis and a moderately thick dermis. This dermis was still moderately acellular and the histologic pattern of the collagen was unremarkable. No remnant of the bovine lattice was found. Speculation favors biodegradation.

The tissue interface between implant and host was often a moderately narrow zone of vascular fibrous connective tissue. This zone was occasionally very pronounced in some foci but in other foci very minimal. In the latter, the transitional zone from normal host muscle or fat to the implant may consist of only several fibrous strands. The latter finding would indicate that the artificial implant can heal without fibrous scarring and that the artificial dermis does not provoke a dermoplastic response. Thickened zones may be secondary to healing of a partially burn damaged wound in the host bed itself, caused by incomplete excision of all damaged tissue.

The clinical behavior of the artificial skin indicates the degree of success achieved in meeting the design criteria. Although only ten patients are reported, with a relatively short follow-up period (2–6 months) we believe we have sufficient evidence to indicate that the artificial skin, as designed, has provided a satisfactory skin substitute following prompt eschar excision. The extensive area of wound closed, extending in one patient to 60% of the body surface area, and the prolonged period of time this skin substitute was maintained in place gives evidence indicating that physiologic wound closure had been achieved. In the patients reported, there was no infection in the grafted area, and both clinical and histologic investigations confirmed that the artificial skin did not elicit an inflammatory or foreign body response. It is our impression that contraction was minimal and no hypertrophic scar has developed. There was no rejection or histologic evidence of an immunologic reaction. Our original hypothesis stating that the artificial dermis, if designed with the proper physicochemical, biochemical, and mechanical properties, would act as a biodegradable dermal template leading to the synthesis of a "neodermis," has received some confirmation. In the time of observation in both clinical evaluation and histologic study, the connective tissue synthesized by cells invading the artificial dermis is anatomically constructed in a fashion resembling normal dermis and not similar to scar fibrosis. We believe this neodermal structure provides some of the physical and cosmetic properties of normal dermis and has led to the improved functional and cosmetic results seen in this

short period of follow-up. There is, of course, no evidence indicating that the anatomic arrangement with its functional and cosmetic benefits will persist for long periods of time. However, at this time there is no indication that the "neodermis" is altering its beneficial configuration in the direction of scar. If the neodermal function persists, considerable long-term improvement in functional and cosmetic results can be expected following the treatment of deep dermal injury.

Although it might be stated that the grafting of the dermal portion of the bilayer artificial skin was permanent, in the strict sense of the word, this is not accurate. The artificial dermis, although serving as a template for the construction of a synthesized "neodermis," is slowly biodegraded and completely resorbed in several months. The turnover of artificial dermis, however, to some extent, resembles the turnover of normal collagenous structures in dermis, which is a continuous process; and because no further maneuver must be carried out to replace the dermal structure for practical purposes it may be thought of as a permanent dermal graft.

It has always been clear that epidermal cells, themselves, obtained from the patient would provide the optimal epidermal covering. However, the use of a temporary Silastic epidermis provides several important contributions to the clinical use of the intact artificial skin. First, it allows the material to be completely manufactured from nonviable raw materials in industrial batch processes and sterilized for immediate use. Further, because of its completely synthetic nature, simple room temperature storage over long periods of time and the potential for mass production raise the possibility of effective biologic and economic use.

At this time we have used the very thin split-thickness graft to replace the Silastic epidermis at a time of clinical election, usually two to three weeks after excision and artificial skin grafting. It is clear that the effectiveness and technical complexity of the artificial skin usage would be simplified if epidermal cells were implanted in the bilayer material at the time of or close to initial grafting. Animal studies indicate that this, at least, in part, is feasible so that our present techniques of grafting are tentative, awaiting further improvement in the artificial skin itself.

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Discussion

Dr. Boyd Withers Haynes, Jr. (Richmond, Virginia): Dr. Burke has just presented a solution to wound healing that has eluded us in the past.

His study is reminiscent of some of the observations made on allograft rejection, which in the separation process following transplantation may lose epidermis but retain a dermal network over which autograft may be successfully placed, will grow, and function satisfactorily.

Dr. Burke has carried that observation a step farther in the sense of providing a dermal structure that can be biodegraded in place and replaced by new collagen and neovascularity. Apparently, no rejection occurs, as may be the case with allograft.

I would like Dr. Burke to offer us any further information that he may have concerning texture, innervation, and contracture. It is early, I am sure, to have observations about innervation, but any observations about contracture would be of special interest.

Dr. Carl Walter (Boston, Massachusetts): Dr. Burke has extended his initial concept of the aseptic treatment of burns to that of providing a physiologic milieu for the orderly healing of extensive, denuded wounds.

As described in John Mulliken's presentation, a semisynthetic template is provided that entices undifferentiated cells to assume a constructive role in wound repair. Among the essential properties that Dr. Burke lists for his template are surface energy and pore size. Tanzawa, a Japanese polymer chemist, last month described the adaptation of plasma cells (fibroblasts and platelets) to adhere and ultimately coat the surface of various synthetic polymers. Upon adhering, the cells developed filopodia that spread over the hydrophobic surface of various polymers. Pseudopods formed that inter-